EVIDENCE OF WATERBORNE TRANSMISSION OF BLASTOCYSTIS HOMINIS

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Abstract. A cross-sectional study was performed in February 2001 to evaluate the prevalence and risk factors of Blastocystis hominis infection in army personnel who resided in an army base in Chonburi, Thailand. A total of 904 army personnel were enrolled in this study. Short-term in vitro cultivation was used to detect B. hominis in stool samples. In this population, B. hominis was the parasite most frequently found, and was identified in 334 of 904 stool specimens (36.9%). A significant association between B. hominis infection and symptoms was identified that might emphasize the role of B. hominis as a human pathogen. After adjustment for potential confounders, significantly increased risk of being infected with B. hominis was associated with being a private, working in a specific unit, and consuming unboiled drinking water. Thus, waterborne transmission of B. hominis infection was indicated at this army base. However, other modes of transmission cannot be ruled out.

INTRODUCTION

Blastocystis hominis is an obligate anaerobic protozoan found in the human intestine. It has long been described as a commensal organism since most cases of blastocystosis were asymptomatic.1–4 However several studies, have reported B. hominis as a causative agent of gastrointestinal symptoms such as diarrhea and abdominal pain in both immunocompetent and immunocompromized hosts.5–10 Its role in gastrointestinal symptoms has been continuously debated.

Knowledge on the epidemiology of B. hominis infection is also rather limited. Blastocystis hominis has been reported to have a high prevalence in developing countries.11–13 In Thailand, the prevalence of B. hominis carriage in various groups is as high as 10–40%.14–16 It is believed that B. hominis is transmitted via the fecal-oral route. Although routes of transmission such as waterborne, foodborne, and person-to-person have been speculated in several reports, controlled studies have rarely been conducted. In our survey in 1999, we found a high prevalence of B. hominis carriage in the personnel who worked in an army base in Chonburi in an eastern province of Thailand (Mungthin M, Taamasri P, unpublished data). The purpose of the present study was to determine epidemiologic and clinical characteristics associated with B. hominis carriage in these army personnel.

MATERIALS AND METHODS

Study population. The research protocol was reviewed and approved by Ethical Committee of the Medical Department of the Royal Thai Army. This cross-sectional study was undertaken at the army base in Chonburi, Thailand in February 2001. The study population consisted of 1,922 army personnel including privates, noncommissioned officers, and officers between 18 and 60 years old. Informed consent was obtained from the enrolled army personnel.

Stool collection and examination. Stool specimens were examined for intestinal parasites immediately after collection by a wet smear preparation in saline and staining with Lugol’s iodine solution. All specimens were then processed for the formalin/ethyl-acetate concentration technique. Since the simple smear and concentration technique is rather insensitive for the detection of B. hominis,17 short-term in vitro cultivation was performed for each stool sample in the medium of Jones supplemented with 10% horse serum.18 The cultures were incubated at 37°C for 48–72 hours and then examined by light microscopy with 10× and 40× objectives. A B. hominis-positive specimen was defined as the identification of any form of B. hominis, i.e., vacuolar, granular, multivacuolar, and cystic forms by short-term in vitro cultivation. Each stool specimen was examined for Cryptosporidium spp. and microsporidia using modified acid-fast and gram-chromotrope staining, respectively. All samples were also tested for common bacterial pathogens that may cause gastrointestinal symptoms. Stool samples were transferred in transport media and cultured for bacterial pathogens using conventional cultivation and identified by biochemical methods at the Department of Microbiology of the Phramongkutklao College of Medicine in Bangkok, Thailand.

Questionnaires. To determine the risk factors and outcomes of B. hominis infection, standardized questionnaires concerning demographic data, sanitary behaviors including cooking and eating habits, source and treatment method of drinking water, pets or animal contact, and a history of present gastrointestinal symptoms were used in this study. Diarrhea was defined as a change in their normal pattern of bowel movements and at least three loose stools during a 24-hour period. Dysentery was defined as at least one passage of mucous bloody stool in one day. The enrolled army personnel were asked to complete the questionnaires when they provided stool specimens.

Statistical analysis. The association between potential risk factors and B. hominis carriage was assessed by the chi-square test with a 95% confidence interval. Univariate analysis was performed using Epi-Info version 6.04b (Centers for Disease Control and Prevention, Atlanta, GA). Odds ratios with 95% confidence intervals and P values were calculated to compare outcomes among study groups. Logistic regression using SPSS for Windows version 9.6 (SPSS, Chicago, IL) was performed for multivariate analysis to assess the independent association of risk factors and B. hominis.

RESULTS

Of 1,922 army personnel in the camp, 904 (47%) persons were voluntarily enrolled in the study. The response rates among officers, noncommissioned officers, and privates were 45.6%, 45.4%, and 48.2%, respectively. The main reason for
Intestinal parasitic infection in 904 army personnel in Thailand

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocystis hominis</td>
<td>337</td>
<td>37.3</td>
</tr>
<tr>
<td>Hookworm</td>
<td>35</td>
<td>3.9</td>
</tr>
<tr>
<td>Strongyloides stercolis</td>
<td>26</td>
<td>2.9</td>
</tr>
<tr>
<td>Giardia intestinalis</td>
<td>24</td>
<td>2.6</td>
</tr>
<tr>
<td>Non-pathogenic amoeba</td>
<td>24</td>
<td>2.6</td>
</tr>
<tr>
<td>Opisthorchis viverrini</td>
<td>17</td>
<td>1.9</td>
</tr>
<tr>
<td>Small intestinal fluke</td>
<td>13</td>
<td>1.4</td>
</tr>
<tr>
<td>Balantidium coli</td>
<td>6</td>
<td>0.7</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>Taenia spp.</td>
<td>2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Clinical outcomes of B. hominis infection. There were 31 patients who had dysentery during our study. Of these, 18 patients (58.1%) were positive for B. hominis by in vitro cultivation. The prevalence of B. hominis infection in the symptomatic group was significantly higher than in the asymptomatic group, of which 36.2% (313 of 864) were positive for B. hominis (P = 0.021, by chi-square test). All specimens were cultured for bacterial pathogens that could be possible causes of gastrointestinal symptoms. Of 31 specimens from dysenteric patients, only one specimen was positive for Salmonella.

Characterization of persons with B. hominis infection. The characteristics of the enrolled personnel and prevalence of B. hominis infections are shown in Table 2. All of the enrolled personnel were men and almost 60% were privates between 21 and 24 years old. These privates had been conscripted from different parts of Thailand and inducted to work at this army base for 6–24 months. The majority (85%) of these privates had original areas of residence in the northeastern and eastern provinces of the country. After induction, these privates were randomly assigned to each working unit including Headquarters, First Battalion, Second Battalion, and Third Battalion. Working and living areas of each unit were totally separated from the others. Food provided for these privates was prepared by their mess section. Sources and treatment methods of drinking water were different among working units. Since most of the privates restrictively worked and lived in each battalion, they always consumed food and drinking water provided by their own battalion. Nearly all of noncommissioned officers and officers lived in or around this army base. Those who lived at the army base were staying in separate houses. They could choose their own food and drinking water.

Prevalence of B. hominis infection was significantly different among different ranks (P = 0.0001), age group (P = 0.006), and working units (P = 0.035). There was no statistically significant difference among those with different original areas of residence, current living areas, and education (Table 2).

Risk factors of B. hominis infection. The results of univariate and multivariate analysis of risk factors and B. hominis infection are shown in Table 3. Univariate analysis showed that army personnel between 21 and 30 years old and privates were approximately 1.7 and 1.6 times at greater risk of acquiring B. hominis compared with the others. Personnel who worked in the Second Battalion had a 1.7 times lower risk of acquiring the infection than those who worked in the other battalions. Consuming boiled water also had protective effect against acquiring blastocystosis.

Multivariate logistic regression analysis showed that private group, working in the Second Battalion, and type of drinking water were independently associated with blastocystosis. Privates were 1.8 times at greater risk of acquiring blastocystosis than those of other rank. Compared with other units, those who worked in the Second Battalion had a two-fold lower risk of acquiring blastocystosis. In addition, those who consumed boiled water had a two-fold lower risk of acquiring blastocystosis than those who did not. There was no significant association between B. hominis carriage and original areas of residence.
dence and current living areas. In addition, there was no significant association of B. hominis infection among groups with regard to education, sanitary behaviors including cooking and eating habits, pets, or animal contact.

**DISCUSSION**

Asymptomatic infection with B. hominis appears to be common. Thus, the ability of B. hominis to cause disease is doubtful. However a number of reports have suggested that B. hominis could be the causative agent of a variety of gastrointestinal diseases such as diarrhea, enteritis, colitis, and irritable bowel syndrome. In addition, B. hominis infections, both with and without severe symptoms, have been reported in immunocompromized patients with various disorders, e.g., acquired immunodeficiency syndrome, poorly controlled diabetes, and leukemia. A recent study by Cirioni and others showed a high prevalence of blastocystis in human immunodeficiency virus–infected patients. The gastrointestinal symptoms in these patients were significantly associated with CD4+ cell counts. Data in the present study might support B. hominis as a human pathogen. A statistically significant association between B. hominis infection and the present history of dysentery was found in the enrolled army personnel. Common clinical symptoms of B. hominis infection, such as diarrhea, abdominal pain, constipation, anorexia, and flatulence, were nonspecific. Although B. hominis-infected patients with dysentery and a clinical picture of colitis have been reported, this clinical findings are not as common as the others. We are aware that this significant difference might be due to co-infections with other intestinal pathogens. However, we have ruled out those bacterial pathogens that could cause dysentery.

Most of army personnel with B. hominis infection in this study were asymptomatic, which is similar to those previously reported in the literature. Many hypotheses have been put forward to explain the different outcomes of the infection. Genetic diversity in B. hominis has been shown in several studies. An association between subgroup and pathogenicity has been postulated; however, there is still no conclusive evidence.

Epidemiologic data of B. hominis infection has been inconclusive in several aspects, particularly the source of infection and mode of transmission. It is assumed that B. hominis is transmitted via the fecal-oral route similar to other intestinal protozoa. Compared with other forms of B. hominis, cysts are more resistant to the environment and proved to be infective in an animal model. Thus, it is suggested that the cystic stage is responsible for transmission. Waterborne transmission has been speculated as the mode of transmission of B. hominis in several studies, especially those conducted in tropical countries and in travelers who just returned from these countries. These travelers also gave a history of consumption of untreated water while they were abroad. In the present study, we have provided additional supportive evidence of waterborne transmission. Using multivariate analysis, we found that those who consumed unboiled water had higher risk of acquiring a B. hominis infection. This result is consistent with our previous study conducted in an army base located in a different province. We found a 2.7 times greater risk of B. hominis infection in those army personnel who consumed untreated water. This finding provides strong evidence for the role of waterborne transmission in B. hominis infection.

The results in this study also indicated that some common methods of water treatment might not be suitable to eliminate B. hominis, especially the cystic stage. Cysts of B. hominis are rather small (3–5 μm) compared with cysts of other common protozoa such as Giardia and Entamoeba. Thus, it is likely that cyst of B. hominis could escape the conventional water filtration techniques. Moreover, it was shown that cysts of B. hominis was resistant to chlorine at the standard concentration used in tap water. Additional evidence that might suggest waterborne transmission of B. hominis in this army base was the reduced risk of getting the infection in the army personnel who worked in the Second Battalion. In this battalion, drinking water was treated by a different method. It involved both carbon block filters and ultraviolet light. In addition, regular replacement of the carbon filter and ultraviolet lamp was done (Taamasri P, unpublished data).

Another risk factor for infection with B. hominis in this army base was being a private. The high prevalence of B.
hominis infection in privates was also shown in our recent studies.14,16 These privates most likely acquired this infection after the induction since they were randomly assigned to various working units and we demonstrated the independent risk factor of being in the Second Battalion after the induction. These privates in each working unit shared their own common activities and environments such as living conditions, military training, sources of food, and drinking water. Thus, the infection might have transmitted via human-to-human contact, contaminated food, or drinking water.

Animals have been speculated to be a source of B. hominis transmitted to humans because Blastocystis-like organisms have been identified in a wide range of animals.31–34 Moreover, a higher risk of B. hominis infection was found in people with closed animal contact.35 A recent study of the full-length small subunit ribosomal DNA (ssu rDNA) sequence of Blastocystis isolated from different hosts has shown a high identity between those of human and pig isolates.36 Consistent with this study, we used B. hominis isolated from the army personnel enrolled in the present study and genetically compared them with Blastocystis isolated from pigs and a horse raised on this army base using a restriction-fragment-length polymorphism analysis of partial ssu rDNA.37 Our study has shown similar banding patterns in some B. hominis isolates, the horse isolate, and all pig isolates. Sequence and phylogenetic analyses have also shown that isolates of Blastocystis from a pig and a horse were monophyletic and closely related to B. hominis. Taken together, these data support the possibility of zoonotic potential of Blastocystis. However, epidemiologic studies at this army base could not find the linkage between animal-to-human transmission and B. hominis infection.

In conclusion, B. hominis infection is the most common intestinal protozoa found in this community. Infection with B. hominis at this army base was associated with dysentery. Our data suggest waterborne transmission of B. hominis infection at this army base. The infection was also common in those who shared common activities and environments. However, other modes of transmission such as human-to-human, animal-to-human, and foodborne cannot be ruled out.

Received July 2, 2003. Accepted for publication October 17, 2003.

Financial support: This work was supported by the Thailand-Tropical Diseases Research Program (T2) (ID 00-1-HEL-24-011).


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